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Personal identification of cold case remains through combined contribution from anthropological, mtDNA and bomb–pulse dating analyses.

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ABSTRACT:

In 1968, a child's cranium was recovered from the banks of a river in Northern Canada. For more than 35 years, the remains were held in trust in a local museum until the 'cold case' was re-opened in 2005. The cranium underwent re-analysis at the Centre for Forensic Research, Simon Fraser University using recently developed anthropological, radiocarbon and genetic techniques. Anthropological analysis of the biological tissues, including craniometrics, skeletal ossification and dental formation and eruption indicated an age-at-death of 4 ±1 years. Radiocarbon dating of enamel from two teeth using a 'bomb-pulse' approach indicated the child was born between 1958-1962. While mini-STR analysis produced marginal results due to poor DNA preservation, analysis of amelogenin gene indicated that the cranium was male. A mitochondrial profile of the HVI and HVII regions was also obtained, and demonstrated a match between the child's remains, and a living maternal relative of the presumed missing child. The results obtained from these multi-disciplinary forensic analyses were consistent with those of a missing child reported from the region in 1965, and resulted in a legal identification 41 years after discovery of the remains. This case study highlights the enormous potential of combining radiocarbon dating with anthropological and mtDNA analyses in producing confident personal identifications for forensic cold cases dating to within the last 60 years.

In 1968, a hunter found a partial cranium on the banks of a river in Northern Canada. The cranium had initially been linked to a four-year-old boy who had been presumed drowned in the river in 1965. The anthropological assessment conducted in 1969 precluded a match with the missing child due to an error concerning age-at-death. The partial cranium had an erupted upper right second deciduous molar, which was mistakenly identified as a permanent first molar, resulting in a gross overestimation of age-at-death (i.e. a child of 7-9 years). Based on this error, the anthropologist concluded that "we are inclined to the belief that the cranium which we have examined is not part of the skeletal system of the missing child" (anonymized letter April 2, 1969 to RCMP).

For more than 35 years, the remains were held in trust by a local museum until new techniques would be available to identify the child and repatriate the remains. In 2005, the remains were submitted to the Ancient DNA Laboratory, Department of Archaeology, Simon Fraser University for multi-disciplinary scientific analysis in a renewed attempt to identify the child. Our analyses were deliberately conducted without background knowledge of the case as provided above. Our aim was to see if any mtDNA existed in the hard tissues, and if so, to advise the authorities of the feasibility of seeking surviving family members for further testing. The anthropological assessment of the hard tissues was also repeated, and associated botanical materials were analyzed for evidence as to geographic origin and seasonality. Given the elapsed time since death, we further sought to determine if application of the new technique of bomb-pulse dating might help determine when the child had been born and provide further indirect evidence of identity.

Methods and Materials

Anthropological Analysis

The remains included an incomplete, dry skeletonized cranium with one remaining erupted tooth (5-5, FDI nomenclature) and several unerupted, forming permanent teeth from the upper right maxilla (Fig. 1). There was postmortem rodent gnawing on many edges of the cranium. The primary means of analysis was visual inspection. Craniometrics were recorded using standard equipment, and photographs were taken of the cranium and adherent plant matter using a Nikon D100 camera and a Keyence VHX-100 Digital Microscope. Methods for age-at-death determination included dental formation, skeletal ossification, and circumference of the cranium. Radiographs were taken of the developing dentition (Fig. 2) using a H.G. Fischer model FP200 portable X-ray unit at SFU to determine age at death. We used standards of tooth formation for both native (1) and non-native children (2) as both biological populations are represented among missing persons in northern Canada. Plant matter inside the neurocranium was observed and removed for identification in an effort to determine the season and location of deposition. This material consisted of a leaf and petioles (Fig. 3).

Radiocarbon Analysis

Recently, radiocarbon dating of tooth enamel has proven to be a remarkably accurate indicator of year-of-birth for forensic cases dating to within the last 60 years (3-6). Above-ground nuclear weapons testing between 1955 and 1963 doubled the natural amount of radiocarbon (^{14}C) in the atmosphere, which has since declined after the test-ban treaty in 1963 (7-11). Since living organisms naturally incorporate atmospheric

carbon atoms into their tissues through the food chain, the bomb-pulse dating techniques can measure the amount of ^{14}C in tooth enamel to indicate what year the tooth was formed, providing that the individual was born within the last 60 years (3-6). Typically, enamel from two teeth formed during different years are analyzed in order to distinguish whether the individual was born before or after the peak of the bomb tests, i.e. to distinguish whether the ^{14}C measurement relates to the rising or falling part of the bomb-pulse curve.

Two teeth from the cranium (5-5, deciduous upper second molar; and 1-5, permanent successional second upper premolar) were prepared at the Department of Cell and Molecular Biology, Medical Nobel Institute, Stockholm, Sweden and radiocarbon dated at the Center for Accelerator Mass Spectrometry , Lawrence Livermore National Laboratory, Livermore, California. The methods for enamel preparation and accelerator mass spectrometry (AMS) sample measurement and analysis are described in (4) and (3) with slight adjustments to the scientific protocol to address the problem of incomplete mineralization of the upper right second premolar crown in this young child. Enamel purification by sonication in 10N NaOH was reduced from 3-5 days to 15 minutes. All other procedures were as described previously. The acid pretreatment to remove the outer surface of the enamel exposed to the base used for enamel purification was also adjusted due to the softer enamel in the deciduous tooth and incomplete mineralization of the permanent tooth. Pretreatment used 0.25N HCl rather than 1.0 N HCl for both teeth and exposure was reduced to 20 minutes for 1-5. All other AMS procedures were as described in (3).

DNA Analyses

The hard tissue samples were analyzed in the Ancient DNA laboratory in the Department of Archaeology at Simon Fraser University using a modified silica spin column method for the retrieval of degraded DNA (12). The Ancient DNA laboratory is specifically designed for and dedicated to ancient DNA analysis, and follows strict contamination control protocols including the use of protective clothing, and the separation of the pre- and post-PCR work. DNA was extracted from two unerupted teeth (CC1: permanent upper first premolar (1-4), and CC2: permanent first upper molar (1-6)) as well as a 2.41g portion of the skull (CC7: right occipital squama). The tooth and bone samples were chemically decontaminated through immersion in 100% commercial bleach (6% sodium hypochlorite) for 7 min, 1N HCl for 1 min and 1N NaOH for 1 min, followed by UV irradiation for 60 min. The tooth and bone samples were crushed into powder using a cryogenic mill, and the tooth or bone powder was incubated overnight at 50ºC in 3-5 ml of lysis buffer (0.5M EDTA, 0.25% SDS, and 2.0 mg/mL proteinase K) in a 15 mL tube. After centrifugation, the supernatant was transferred to an Amicon centrifugal filter device, Ultra-4 (Millipore, Billerica, MA). The extract was concentrated to less than 100 µL and then purified using QIAquick columns (Qiagen, Valencia, CA), from which approximately 100 µL of DNA solution were collected for each sample.

The analysis initially targeted mitochondrial DNA (mtDNA), which is present in higher copy numbers than nuclear DNA, making it easier to recover from degraded remains. PCR amplifications were conducted in a Mastercycler Personal (Eppendorf, Hamburg,

Germany) in a 30 μL reaction volume containing 50 mM KCl, 10 mM Tris-HCl, 2.5 mM

$MgCl_2$, 0.2 mM dNTP, 1.0 mg/mL BSA, 0.3 μM each primer, 3.0 μL DNA sample and

2.5 U AmpliTaq Gold™ (Applied Biosystems). PCR was run for 40 cycles at 94°C for 30

seconds (denaturing), 52°C for 30 seconds (annealing), and 72°C extension for 40

seconds, with an initial 12 minute denaturing period at 95°C. The samples were amplified

using multiple overlapping primer sets designed to amplify fragments of the

hypervariable regions (HVI and HVII) of the human mitochondrial DNA genome (13).

Extraction blanks and PCR negative controls were carried out for each PCR experiment.

Five μL of PCR product were visualized via electrophoresis on a 2% agarose gel using

SYBR Green™ staining. The remainder of the PCR products were purified using

MinElute™ purification kits (Qiagen, Valencia, CA) and sent to Macrogen Inc. for

sequencing. The obtained electropherograms were edited, aligned and compiled using

ChromasPro software (www.technelysium.com.au). The consensus mtDNA sequence

obtained from multiple overlapping PCR amplifications was compared to mtDNA

profiles from laboratory personnel, the SWGDAM database, and later to a putative family

member. To determine the sex of the individual, the DNA extracts from CC1 and CC2

were also amplified using primers designed to target short fragments of the amelogenin

gene on the X and Y chromosomes (14, 15).

mtDNA Analysis of Putative Relatives

Later in our investigations, a maternal DNA reference from a putative sister was obtained

through a buccal swab. The DNA from the living relatives was analyzed only after a

complete and replicated mtDNA profile was obtained for the cranium in order to avoid

the possibility of contaminating the 'cold case' remains with the modern reference DNA

sample (16). The FTA card underwent two separate DNA extractions (FC1, FC2), in the Modern DNA laboratory at the Centre for Forensic Research at Simon Fraser University. The FTA card was extracted using the Qiagen QIAamp DNA Investigator Kit following the manufacturer's instructions. The HVI and HVII regions of the mtDNA control region were targeted using two primer sets (**mt1-1** 5'- TAACTCCACCATTAGCACCC-3', **mt2-2** 5'-TGATTTGAGGGAGGATGGTG-3' and **mt3-1** 5'- CACCCTATTAACCACTCACG-3', **mt4-2** 5'-GCTGGTGTTAGGGTTCTTTG-3') as described in (17). The fragments were amplified using the PCR conditions listed above.

The preliminary mtDNA match observed between the cranium and the maternal reference, and determination of male sex of the cranium, led to the next step of seeking a paternal DNA reference from the putative father. It was expected that nuclear STR profiling of two samples would provide sufficient discriminative power to evaluate presumed paternal relationship. The paternal reference (buccal swab) was shipped to Yang's Lab for DNA extraction (FC5) in the Modern DNA Laboratory at the Centre for Forensic Research at Simon Fraser University, using the methods listed for the maternal reference above.

Aliquots of the DNA extracts from the remains (teeth and cranium) and putative relatives (father and sister) were sent to the Forensic DNA Laboratory within the Centre for Forensic & Security Technology Studies (BC Institute of Technology, Burnaby, BC) for mini-STR profiling. All samples were quantified using *Applied Biosystems Quantifiler^{TM}* (Foster City, CA), a human DNA quantification kit using an *Applied Biosystems 7500*

Real-Time PCR System. The manufacturer's protocol was followed for all samples except CC1 and CC2, which had 1 µL of sample and 1 µL distilled water (instead of 2 µL of sample).

Approximately 35pg of DNA from the cranium (CC7) extract sample was amplified in duplicate using *Applied Biosystems AmpFlSTR MiniFiler*^{TM} kit in a 12.5 µL reaction. Similarly, 200pg of DNA from each familial sample was amplified. PCR was performed in a *Perkin Elmer 2400* thermal cycler. Fragment analysis was performed on an *Applied Biosystems 310 Genetic Analyzer* using *GeneMapper ID* (v3.2). Genetic analysis samples were prepared in deionized formamide (1.5µL *MiniFiler*^{TM} PCR product in 25µL of formamide/GS-500 LIZ). All other conditions were as per the manufacturer's suggested protocol.

Results and Discussion

Elapsed Time since Death Based on Taphonomic Indicators

The cranium was devoid of soft tissue and grease, and had acquired discoloration from prolonged contact with the soil. Several areas of the cranium had been gnawed by rodents (Fig. 1). According to Klippel and Synstelian (18), in Tennessee, only those human remains that have been exposed for more than two years demonstrate gnaw marks, providing a minimum estimate for elapsed time since death. In a separate forensic case involving a child's cranium from a similar region of Canada, remnants of nasal cartilage were still preserved after eight years, though the bones were more severely exfoliated (19). Based on these (weak) indicators, a rough estimate of elapsed time since death was judged to be 5 to 10 years prior to discovery. As the cranium was recovered in 1968,

death was estimated to have occurred between 1958 and 1963.

Results of Anthropological Analysis

The ancestry or sex of the child could not be determined using conventional anthropological techniques since the individual was still very immature at death. There were no discrete traits of the available skeleton or teeth that might suggest First Nations ancestry (e.g., no enamel extensions, the oval window is visible, lambdoidal suture lacks wormian bones) (20).

Personal identification of a child is greatly assisted by an accurate and precise age-at-death determination. In this case, with only a cranium and partial upper dentition preserved, our options for determining age-at-death included: a) dental formation; b) skeletal ossification; and c) head circumference, with the results of the analyses summarized on Table 1.

Dental formation: A radiographic evaluation (Fig. 2) of four forming right maxillary permanent teeth using native Canadian dental formation standards (1) yielded an age-at-death estimate of 4.0 years (ranging from 3.19 to 5.45 years; both sexes considered). Later, the removal of tooth 5-5 from the dental crypt revealed its incomplete root apices (Figure 4). The tooth 5-5 root apices were judged to be at stage $>A^{\frac{1}{2}}$. For children of European ancestry, stage $A^{\frac{1}{2}}$ is typically is obtained for the isomere of this tooth at about 2.3 to 2.4 years, while the apex is complete between 2.8 to 3.1 years $\pm$ 0.5 years [male and female, respectively] (2). Root apex formation is typically considered a more precise

estimate of dental formation than obtainable through radiographs alone (21); this tooth alone indicated an age-at-death of around 2.35 to 2.95 (±0.5 years).

Skeletal ossification: The basi-occipital component of the occipital bone, shown in Fig. 1 is only partially fused to the rest of the occipital bone, indicating an age less than 5-6 years (22). Its dimensions (breadth =32.1mm, length 19.41mm) indicate an age greater than 4 years, 7 months (23).

Head circumference: The cranial circumference was 495mm, which was increased to 520 mm to allow for the missing soft tissue (24). Jones (25) provides head circumference measurements for children up to the age of 3 years; according to these criteria, this cranium falls within the 90-95th percentile for children aged 36 months (25). Based on cranial circumference, the age of the child is greater than 3 years.

In sum, dental and skeletal age indictors centre upon an age-at-death of 3.85 ±1 year (i.e, 2.85 to 4.85 years) (Table 1). For discussion purposes, the age-at-death was assumed to be 4±1 years.

Results of Botanical Analysis

The interior of the cranium yielded several botanical remains, the most diagnostic of which included four bud scales closely resembling 'balsam poplar' or black cottonwood (*Populus balsamifera*) (Fig 3). Based on number and preservation of the leaf buds found within the cranium, poplar trees were likely growing within the depositional environment

(provided that the leaf buds were introduced into the cranium through natural means such as wind or water). However, due to the widespread distribution of the balsam poplar, the botanical analysis could not provide any specific geographic information for the remains. The plant matter suggests that the inside of the neurocranium became accessible for the deposition of plant material from nearby Poplar trees in the Spring season.

Results of Radiocarbon Analysis

This case yielded radiocarbon values for two teeth (see Fig. 5). Tooth 5-5 (deciduous upper second molar) possessed a ^{14}C/C concentration of 1.206±0.005 (Fraction modern) indicating that the average of the crown's enamel formation span occurred between 1959 and 1961.7. Tooth 5-5's crown forms over a period from 11 weeks prenatal to 6-11 months postnatal (19, 26, 27). Thus, a reasonable average estimate for the ^{14}C/C value from tooth 5-5 may be 1.5 to 6.5 months after birth, which is consistent with a birth year between 1958 and 1962.

The enamel from tooth 1-5 (permanent upper second premolar) yielded a ^{14}C/C concentration of 1.553±0.006 (Fraction modern) indicating an average of enamel formation of 1963. Tooth 1-5 was incomplete at the time of death, indicating that death occurred in or after 1963. Given the age-at-death estimate of 3-5 years (see above), the ^{14}C values for tooth 1-5 are also consistent with a year of birth between 1958 and 1960.

The ^{14}C/C concentrations of tooth 5-5 and tooth 1-5 intersect on both the ascending and descending slopes of the bomb-pulse curve. However, the average dates of enamel formation on the descending portion of the curve would be 1984.1 to 1985.8 for tooth 5-

5, and 1968.8 to 1970.7 for tooth 1-5. In that the deciduous tooth deposits its enamel before its successor, and considering the remains were recovered prior to 1984, it is clear that the later dates based on the descending portion of the bomb-pulse curve should be rejected.

In sum, the ^{14}C dates in combination with the age-at-death estimate using anthropological techniques suggest that the child represented by the cranium was born between 1958 and 1962, and died between 1963 and 1968.

Results of mtDNA and Amelogenin Analysis

The initial analysis focused on the hypervariable regions (HVI and HVII) of the mtDNA control-region. More than 16 separate overlapping amplicons were sequenced to produce a consensus profile for the cranium, resulting in a total of 760bp of control region mtDNA. The obtained profile covered portions of both the HVI (16017-16411) and HVII (15-381) regions: A73-G, A263-G, T16224-C, T16311-C (as compared with the revised Cambridge reference sequence (28)). Based on mutations T16224-C and T16311-C, the obtained mtDNA profile can be designated as haplogroup K, a haplogroup primarily found in European populations (29, 30). The mtDNA profile has no match among 4839 individuals in SWGDAM database (31) and the four closest sequences (with one base pair difference) are from Caucasian populations.

The authenticity of the obtained sequences was secured by multiple criteria, including: (i) the use of dedicated ancient DNA facilities equipped with UV filtered ventilation,

positive airflow, and bench UV lights; (ii) a vigorous decontamination protocol of the bone samples before DNA extraction; (iii) the inclusions of blank extracts and PCR negative controls; (iv) DNA sequences obtained from different tooth/bone extracts, as well as sequences obtained from multiple amplification of the same extract all yielded the same sequences; (v) the modern reference sequences were processed only after the analysis of the cranium had been completed; and (vi) the cranium's mtDNA profile differed from that of DNA laboratory personnel and the forensic anthropologist by at least two base pairs.

In addition to mtDNA analysis, the DNA extracts were also amplified with primers designed to target short fragments of the amelogenin gene on the X and Y chromosomes. While the CC1 DNA extract failed to amplify, both the CC2 and CC7 extracts amplified the Y-chromosome amelogenin fragment, indicating that the cranium belonged to a male individual.

Results of Complete Biological Profile

These analyses, which were conducted initially without knowledge of the putative identity or date of disappearance, produced the following biological profile for the cranium: a male, most likely of European ancestry, aged 4+/-1 year, born in the interval from 1958 to 1962 and who died between 1963-1968. His remains were sufficiently skeletonized that plant matter entered the skull in the Spring season.

Correspondence with the authorities indicated that there was a boy of European ancestry who had gone missing (and presumed drowned) in the area of the cranium's discovery in

early 1965, aged 4.63 years. Based on the correspondences between the cranium and the missing child, a maternal relative was contacted to provide a mtDNA reference for comparison.

Comparison of mtDNA with putative family references

The two DNA extracts from the maternal relative (FC1, FC2) both produced the same mtDNA profile as the cranium, amplifying 976bp of mtDNA from the HVI (15973-16569) and HVII (1-381) regions: A73-G, A263-G, T16224-C, T16311-C. The maternal reference also displayed a mutation at position T16519-C, a region that could not be amplified from the cranium DNA extract. The profiles match at all other polymorphic areas.

In addition to the sibling, a paternal reference was also sought in order to add further evidence in support of the child's identity through mini-STR analysis. The quantification of the familial reference samples yielded sufficient DNA for STR typing and gave full profiles suitable for comparison (data not shown). The two dental extracts (CC1, CC2) yielded no quantifiable nuclear DNA, however the cranial sample (CC7) yielded approximately 7 pg/uL of nuclear DNA. An attempt was made to amplify the limited nuclear DNA recovered in this sample in duplicate using *AmpFlSTR MiniFiler^{TM}* kit. Mini-STR's have been shown to be effective for amplifying DNA from degraded human remains (32). Previously, an internal validation study conducted at BCIT determined that the optimal DNA input amount for the *MiniFiler^{TM}* kit is 200 pg for a reaction volume of 12.5 µL (half of manufactures recommended volume). This validation study also tested a lower limit of DNA input down to 62 pg, which was successfully amplified using a

variety of DNA sample types (data not shown). STR-typing was approached with extreme caution in this case given the low level (35pg) of input DNA. During the interpretation phase, it was apparent that the DNA profiles generated for CC7 contained extreme dropout due to stochastic effects (Table 2). Although limited conclusions can be drawn from the STR data, the presence of a Y allele indicated the remains are that of a male. Five loci yielded peak data although some peaks were subthreshold. Of these, two loci (D18S51 and D2S1338) yielded a heterozygote peak pattern although in different replicates (data not shown) and in each, an obligatory paternal allele was present, consistent with a parent-child relationship. One locus (FGA) lacked an obligatory paternal allele although there was only a single, subthreshold allele present in one of the replicates consistent with dropout. If one utilizes the four loci in agreement between the child and putative father (omitting FGA), a combined likelihood ratio of 14 is estimated (based on the Caucasian STR allele database) and represents moderate support in favor of an identification.

Despite the minimal STR data in support of the identity of the missing child, the biological profile developed through anthropological, radiocarbon dating, and the along with the mtDNA profile match with the maternal relative provided the necessary evidence to secure a legal identification for the remains 41 years after the initial discovery.

Combined mtDNA and Radiocarbon Approach

Identification of human remains where greater than 25 years have elapsed since death pose several challenges. First, the DNA in these 'cold case' remains may be severely degraded, and therefore nuclear DNA may not be present in quantities sufficient for optimal STR analysis, as occurred in the case study presented here. Additionally, even when nuclear DNA is preserved, it may be difficult to locate or obtain a DNA reference sample from closely related individuals that would be suitable for comparison. Mitochondrial DNA profiles of the HVI and/or HVII regions are increasingly being used as a method of identification for forensic 'cold cases'. Mitochondrial DNA is present in higher copy numbers per cell than nuclear DNA, thus increasing the chances of retrieving sufficient quantities of DNA for analysis. Additionally, the maternal inheritance pattern of mtDNA often allows for a greater likelihood of locating and obtaining a suitable reference sample from relatives.

However, mitochondrial profiles alone cannot usually provide definitive identification, especially if the mtDNA haplotype is a common one within a particular population. If mtDNA profiles are to be utilized for personal identification in forensic contexts, then it is crucial to know the frequencies of different mtDNA profiles within the populations (33). In mtDNA analyses of British (34), Croatian (35) and Nairobi (36) populations, the presence of common mtDNA haplotypes has ranged from 5-17%, obviously limiting the discrimination power of certain haplotypes for personal identification. Additionally, problems such as heteroplasmy or point mutations may also hinder identifications (30).

While whole mitochondrial genome analysis using SNPs may provide an additional discriminatory power (37), this type of technique may be costly and time consuming. Furthermore, even whole mtDNA genomes may not separate maternal relatives in forensic context such as mass fatalities where siblings or maternal cousins are present within the same death assemblage.

The combination of mtDNA profiles and radiocarbon dating, however, can provide a robust personal identification for those forensic cold cases dating to within the last half century. Since the first molar crown takes less than four years to form starting at birth (38), radiocarbon dating of this tooth can provide an accurate and reasonably precise interval of birth for individuals born after 1955 (4). By dating the enamel of the third permanent molar (the last tooth to form, in the interval between 9 and 12 years of age (38), the interval of birth may be extrapolated for individuals born after about 1943 (4). With an average absolute error of 1.6 years, the radiocarbon method can provide a much more accurate and precise year of birth than traditional anthropological analysis of the skeleton, especially for skeletally mature individuals (4, 39). A precise interval of birth can provide strong corroborative evidence for a personal identification, as well as significantly narrowing down the list of missing persons where there is no presumptive ID. A combined mtDNA profile match and year-of-birth match can provide the evidence necessary for a 'cold case' legal identification, even if the mtDNA haplotype is a relatively common one.

Furthermore, in mass graves or mass fatality contexts, a combined DNA and radiocarbon dating approach provides the additional benefit of distinguishing between maternal relations. When DNA matches are based on matches with relatives (rather than known samples of the victim's own DNA), distinguishing between childless siblings in commingled mass graves can be impossible, even when STR profiles can be generated (40). Though the siblings will have different STR profiles, they cannot be distinguished based on parental DNA references alone. If the siblings are of the same sex and closely related in age, anthropological age-at-death techniques may not be precise enough to discriminate between them, such as in the case of many adolescent brothers killed in the Srebrenica event in Bosnia and Herzegovina (40). Radiocarbon dating of tooth enamel, in combination with STR and/or mtDNA analysis, has the potential to distinguish between siblings even one year apart in age, provided their birth year occurred after 1943.

While the particular anthropological, genetic and radiocarbon techniques discussed above have been applied separately in the past, this case study highlights the enormous potential of these combined techniques for 'cold case' identifications and other challenging forensic contexts dating to the last half century.

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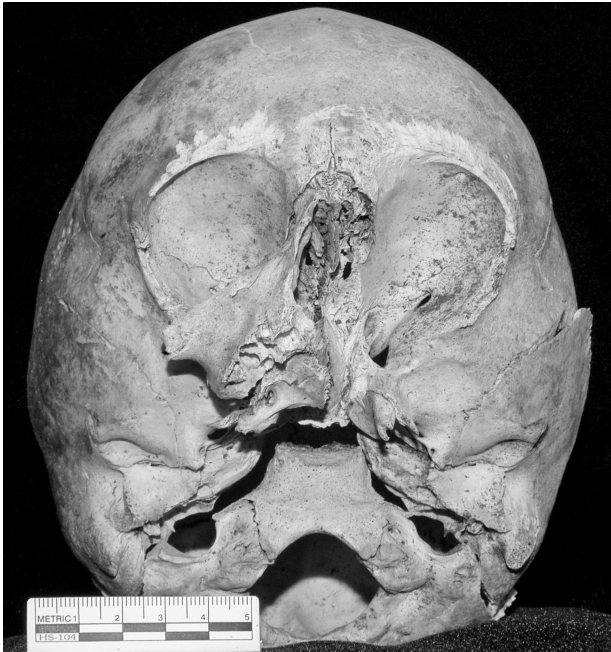
Figures

Fig. 1. Cranium: infero-frontal view after removal of teeth

Fig.2. Radiographic lingual view of right maxillary dentition showing, from mesial to distal, forming canine crown (1-3), forming first premolar crown (1-4), erupted second deciduous molar (5-5), forming un-erupted and tilted second premolar crown (1-5), and forming first molar with root initiation (1-6).
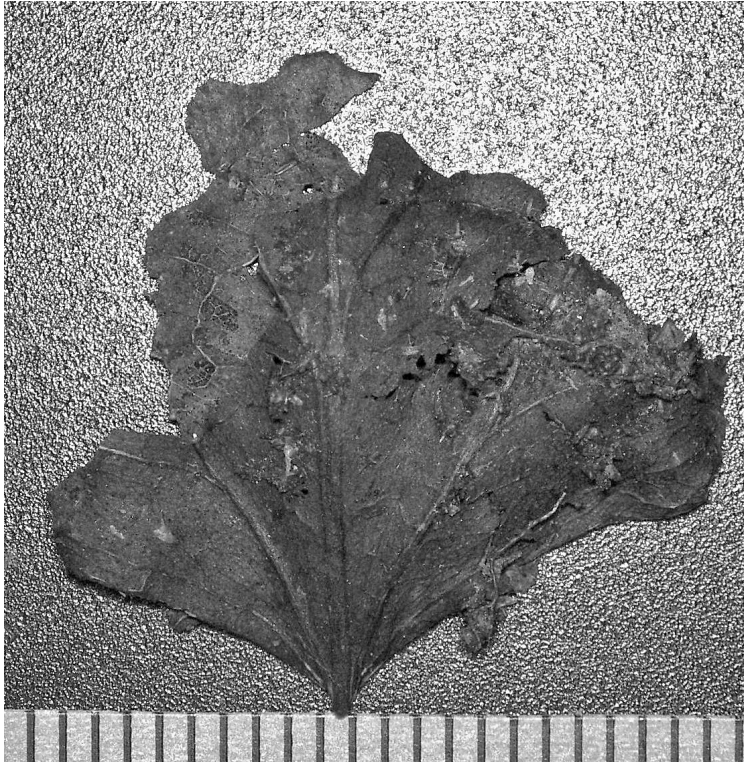
Fig. 3. Plant matter from inside cranium: consistent with being Poplar leaf, bud
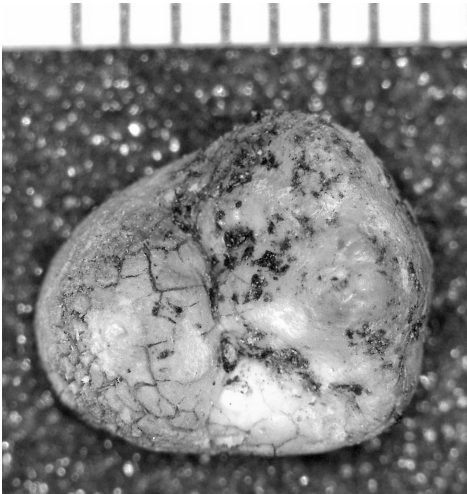scales and petioles (scale mm)

Fig. 4. Dental materials used for age determination and elapsed time since death: upper-buccal view of tooth 5-5 showing incomplete roots; lower-occlusal view of tooth 1-5 showing dessication cracks in this incompletely mineralized premolar crown (scale in mm).
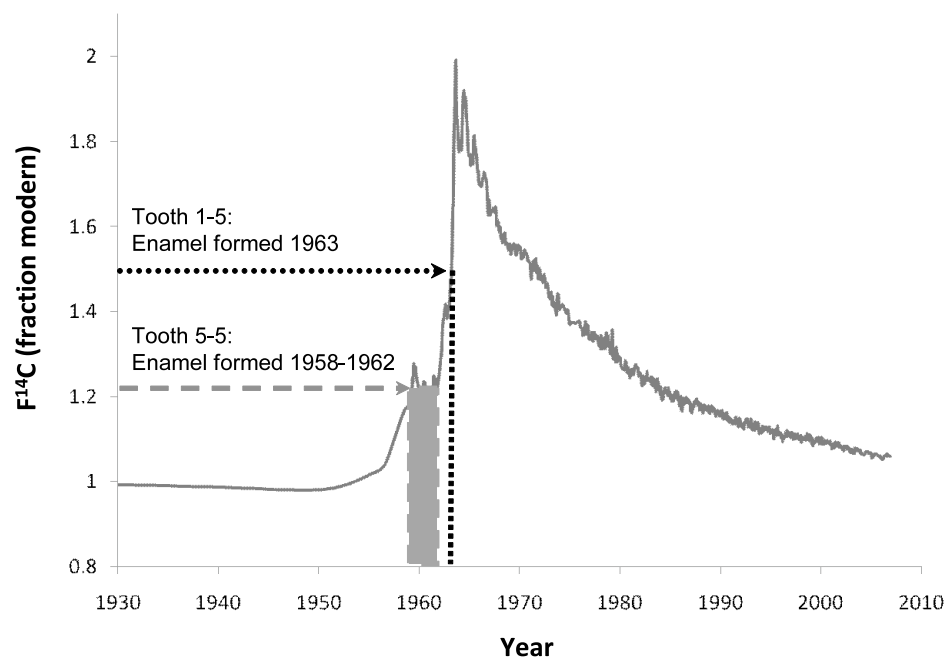
Figure 5   Date of enamel formation based on the ^{14}C/C concentration.. The solid
line indicates the increase in   atmospheric ^{14}C/C caused by above-ground nuclear testing
between 1955-63, and its subsequent decline (9-11). The vertical axis denotes ^{14}C/C
concentration using the F^{14}C nomenclature (7) designed for reporting bomb-pulse data.

TABLE 1 - Summary of age at death indicators

Analytical Technique	Age-at-Death (years)
Stage of tooth formation	3.19-5.45
Stage of root formation	2.35 -2.95 (±0.5)
Basi-occipital size/fusion	4.6-6 years
Head circumference	>3.0
Average age-at-death	**2.85-4.85**

TABLE 2 - Mini-STR Allele Information for the deceased

Sample	D13S317	D7S820	Amel.	D2S1338	D21S11	D16S539	D18S51	CSF1PO	FGA
Cranium (CC7-1)	11	-	-	17,20*	-	-	14	(11)	(18)
Cranium (CC7-2)	11	-	(X),Y	17	-	-	13,14	10,(11)	-

- indicates complete allelic dropout

( ) indicates subthreshold allele (peak < 100 rfu)

Underlined allele indicates obligatory paternal allele present

* Paternal reference had alleles 17,20 at locus D2S1338